

ASCI2
B2
~~The transformed *E. coli* DH5 α containing cosmid pKP4 containing a portion of the~~
Klebsiella genome encoding the glycerol dehydratase enzyme was deposited on 18 April 1995 with the ATCC under the terms of the Budapest Treaty and was designated ATCC 69789. The transformed *E. coli* DH5 α containing cosmid pKP4 containing a portion of the *Klebsiella* genome encoding a diol dehydratase enzyme was deposited on 18 April 1995 with the ATCC under the terms of the Budapest Treaty and was designated ATCC 69790. The *Pseudomonas aeruginosa* strain PAO 2845:pDT9, transformed with a plasmid containing the *dhaB* operon was deposited on 11 April 1996 with the ATCC under the terms of the Budapest Treaty and was designated ATCC 55760. The *Pichia pastoris* strain MSP42.81, transformed with non-replicative plasmids containing expression cassettes for the *dhaB1*, *dhaB2*, *dhaB3* and *dhaT* genes, was deposited on 11 April 1996 with the ATCC under the terms of the Budapest Treaty and was designated ATCC 74363. The *Saccharomyces cerevisiae*, strain pMCK1/10/17(HM)#A, transformed with a plasmid containing the *dhaB1*, *dhaB2*, *dhaB3*, and *dhaT* operon, was deposited before the filing of the instant international application, on May 9, 1996, with the ATCC under the terms of the Budapest Treaty and was designated ATCC 74370. The *Streptomyces lividans* strain SL/14.2, transformed with a plasmid containing the *dhaB1*, *dhaB2*, *dhaB3*, and *dhaT* operon, was deposited before the filing of the instant international application, on May 9, 1996, with the ATCC under the terms of the Budapest Treaty and was designated ATCC 98052. The *Bacillus licheniformis* strain BG188/pM26 (Clone #8), transformed with a plasmid containing the *dhaB1*, *dhaB2* and *dhaB3* operon, was deposited before the filing of the instant international application, on May 9, 1996, with the ATCC under the terms of the Budapest Treaty and was designated ATCC 98051. The *Bacillus subtilis* strain BG2864/pM27 (Clone #1), transformed with a plasmid containing the *dhaB1*, *dhaB2*, *dhaB3* and *dhaT* operon, was deposited before the filing of the instant international application, on May 9, 1996, with the ATCC under the terms of the Budapest Treaty and was designated ATCC 98050. The *Aspergillus niger* strain TGR40-13, transformed with a plasmid containing the *dhaB1*, *dhaB2*, *dhaB3* and *dhaT* operon, was deposited before the filing of the instant international application, on May 9, 1996, with the ATCC under the terms of the Budapest Treaty and was designated ATCC 74369. "ATCC" refers to the American Type Culture Collection international depository located at 10801 University Blvd., Manassas, VA 20110-2209, U.S.A. The designations refer to the accession number of the deposited material.

At page 41, delete the paragraph containing lines 3-8 just before "Example 12", and insert therefor:

B3
ASCI2
~~Transformants containing *dhaT*, *dhaB1*, *dhaB2* and *dhaB3*, constructed as described in Examples 9 and 10, are grown aerobically or anaerobically with shaking at 29 °C in SMM supplemented with 20 mg/L adenine sulfate, 30 mg/L L-lysine, 1 mg/L vitamin B₁₂. Growth~~

Docket No.: CR9715 US DIV (G)

B3
conclude

~~continues until stationary phase is reached and the presence of 1,3-propanediol is determined~~
by HPLC. Transformant *S. cerevisiae* pMCK1/10/17(HM)#A was deposited and designated
~~ATCC 74370.~~

At page 47, delete the paragraph containing lines 20-26 just before "Example 16" and insert therefor:

B4 dacy

~~*S. lividans* TK23/pDT14 (Clone #2) (ATCC 98052, also identified as *S. lividans* strain SL 14.2), inoculated from a TSA plate, was grown in 25 mL of TSB (Tryptone-Soy Broth, Difco, Detroit, MI) plus 1% glucose, 2% glycerol, 1 mg/L vitamin B₁₂, 50 µg/mL thiostrepton in a 250 mL flask. The shake-flask was incubated at 30°C with vigorous shaking for three days, after which 3 mg/L 1,3-propanediol was detected by GC-MS analysis (TMS derivative) in the supernatant as described in GENERAL METHODS.~~

At page 50, delete the paragraph entitled "Production of 1,3-propanediol in recombinant *Bacillus*" and containing lines 26-34, and insert therefor:

Chase
BG

~~*B. licheniformis* strain BG188 transformed with pM26 (Clone #8) (ATCC 98051) was grown in a shake flask containing 25 mL of LB (Difco) plus 1% glucose and 10 µg/mL kanamycin at 30 °C overnight with vigorous shaking, after which 1 mL was used to inoculate 25 mL of LB plus 1% glucose, 1% glycerol, 0.33 µg/mL vitamin B₁₂, and 10 µg/mL kanamycin in a 250 mL flask. Shake flasks were incubated at 30°C with vigorous shaking and after 9 h of growth 300 µg/L 1,3-propanediol was detected by GC/MS (TMS derivitization) as described in GENERAL METHODS.~~

At page 50, delete the paragraph containing lines 35-38 and on page 51, delete lines 1 and 2 and insert therefor:

Chase
BG

~~*B. subtilis* strain BG2864 transformed with pM27 (clone #1) (ATCC 98050) was grown in a shake flask containing 25 mL of LB plus 1% glucose, 1% glycerol, 0.33 µg/mL vitamin B₁₂, and 10 µg/mL chloramphenicol in a 250 mL flask. Shake flasks were incubated at 30 °C with vigorous shaking and after 43 h of growth, 1,3-propanediol was detected.~~

At page 63, delete the paragraph just before Example 23 containing lines 15-17, and insert therefor:

B7 Chase

~~Applicants have deposited a recombinant *Aspergillus niger* strain TGR40-13, comprising a DNA fragment encoding dhaB(1-3), dhaBx and dhaT (ATCC 74369).~~

In the Claims:

Delete Claims 1, 3-5, 7-19, and 32-33.

Docket No.: CR9715 US DIV (G)

2. (Amended One Time) A bioconversion process to produce 1,3-propanediol comprising contacting, under suitable conditions, glycerol or dihydroxyacetone with a single recombinant microorganism having at least one exogenous gene expressing a glycerol dehydratase enzyme, the microorganism selected from the group consisting of members of the genera *Aspergillus*, *Saccharomyces*, *Zygosaccharomyces*, *Pichia*, *Kluyveromyces*, *Candida*, *Hansenula*, *Debaryomyces*, *Mucor*, *Torulopsis*, *Methylobacter*, *Bacillus*, *Streptomyces*, and *Pseudomonas*.

6. (Amended One Time) The process of Claim 2 wherein the microorganism is transformed with at least one exogenous DNA fragment encoding encoding dhaB1, dhaB2, and dhaB3 and /or dhaT.

31. (Amended One Time) A recombinant eucaryote microorganism selected from the group consisting of yeast and filamentous fungi, and expressing an exogenous glycerol dehydratase enzyme.

REMARKS

Applicants regret the confusion as to which claims are currently pending. Claims 1, 3-5, 7-19, and 32-34 have been cancelled herein. Claims 20-30 were previously cancelled in the preliminary amendment. Claims 2, 6, and 31 are currently pending. These claims, as amended, are drawn to the bio-production of 1,3-propanediol by a recombinant microorganism having at least one exogenous glycerol dehydratase gene and using glycerol or dihydroxyacetone as the substrate.

Specification:

Applicants have supplied the ATCC deposit accesssion number information for pages 6 and 7. Accurate directions on the insertion of additional ATCC deposit accession number information on pages 41, 47, 50, 51, and 63 are also provided herein.

Claim 32 has been deleted at the suggestion of the Examiner as being substantially duplicate of allowed Claim 31.

Double Patenting:

Claims 1-18 and 31-33 stand rejected under the judicially-created doctrine of obviousness-type double patenting as being unpatentable over Claims 1-4 of US 6,025,184. Applicants provide herein a terminal disclaimer with regard to Claims 1-8 of the instant case.